

- 1 1. A tissue engineering construct, comprising:
2 embryonic stem cells;
3 a three-dimensional cell support matrix, wherein the matrix is resistant to
4 contractile forces exerted by the stem cells; and
5 at least one growth factor selected to promote differentiation of the stem cells
6 along a predetermined cell lineage or into a specific cell type.
- 7 2. The tissue engineering construct of claim 1, wherein the stem cells are
8 mammalian embryonic stem cells.
- 9 3. The tissue engineering construct of claim 2, wherein the cells are human
10 embryonic stem cells.
- 11 4. The tissue engineering construct of claim 1, wherein the cell support matrix
12 comprises a poly(lactic acid) – poly(lactic acid-co-glycolic acid) mixture.
- 13 5. The tissue engineering construct of claim 4, wherein the cell support matrix
14 comprises a 50/50 mixture of poly(L-lactic acid) and poly(lactic acid-co-glycolic
15 acid).
- 16 6. The tissue engineering construct of claim 1, wherein a cross-sectional area of the
17 matrix is not reduced by more than 50% under a contractile force exerted by the
18 embryonic stem cells.
- 19 7. The tissue engineering construct of claim 6, wherein a cross-sectional area of the
20 matrix is not reduced by more than 40% under a contractile force exerted by the
21 embryonic stem cells.
- 22 8. The tissue engineering construct of claim 7, wherein a cross-sectional area of the
23 matrix is not reduced by more than 30% under a contractile force exerted by the
24 embryonic stem cells.

- 1 9. The tissue engineering construct of claim 8, wherein a cross-sectional area of the
2 matrix is not reduced by more than 20% under a contractile force exerted by the
3 embryonic stem cells.
- 4 10. The tissue engineering construct of claim 9, wherein a cross-sectional area of the
5 matrix is not reduced by more than 10% under a contractile force exerted by the
6 embryonic stem cells.
- 7 11. The tissue engineering construct of claim 10, wherein a cross-sectional area of the
8 matrix is not reduced by more than 1% under a contractile force exerted by the
9 embryonic stem cells.
- 10 12. The tissue engineering construct of claim 1, wherein the cell support matrix
11 further comprises a coating including an agent that promotes cell adhesion.
- 12 13. The tissue engineering construct of claim 12, wherein the agent that promotes cell
13 adhesion is selected from fibronectin, integrins, and oligonucleotides that promote
14 cell adhesion.
- 15 14. The tissue engineering construct of claim 1, wherein the cell support matrix is
16 biodegradable or non-biodegradable.
- 17 15. The tissue engineering construct of claim 14, wherein the cell support matrix is
18 selected from PLA, PGA, PLGA, poly(anhydrides), poly(hydroxy acids),
19 poly(ortho esters), poly(propylfumerates), poly(caprolactones), polyamides,
20 polyamino acids, polyacetals, biodegradable polycyanoacrylates, biodegradable
21 polyurethanes, polysaccharides, polypyrrole, polyanilines, polythiophene,
22 polystyrene, polyesters, non-biodegradable polyurethanes, polyureas,
23 poly(ethylene vinyl acetate), polypropylene, polymethacrylate, polyethylene,
24 polycarbonates, poly(ethylene oxide), co-polymers of any of the above, adducts of
25 any of the above, and mixtures of any of the above polymers, co-polymers, and
26 adducts with one another.

- 1 16. The tissue engineering construct of claim 1, further comprising one or more
2 biomolecules, small molecules, or bioactive agents disposed within the cell
3 support matrix.
- 4 17. The tissue engineering construct of claim 1, further comprising a gel that coats
5 internal and external surfaces of the cell support matrix.
- 6 18. The tissue engineering construct of claim 17, wherein the gel is selected from
7 collagen gel, alginate, agar, and Growth Factor Reduced MATRIGEL™.
- 8 19. The tissue engineering construct of claim 18, wherein the gel further comprises
9 one or more of laminin, fibrin, fibronectin, proteoglycans, glycoproteins,
10 glycosaminoglycans, chemotactic agents, or growth factors.
- 11 20. The tissue engineering construct of claim 1, wherein the growth factor is selected
12 from cytokines, eicosanoids, and differentiation factors.
- 13 21. The tissue engineering construct of claim 20, wherein the growth factor is
14 selected from activin-A (ACT), retinoic acid (RA), epidermal growth factor, bone
15 morphogenetic protein, TGF- β , hepatocyte growth factor, platelet-derived growth
16 factor, TGF- α , IGF-I and II, hematopoietic growth factors, heparin binding
17 growth factor, peptide growth factors, erythropoietin, interleukins, tumor necrosis
18 factors, interferons, colony stimulating factors, fibroblast growth factors, nerve
19 growth factor (NGF) and muscle morphogenic factor (MMF).
- 20 22. The tissue engineering construct of claim 1, wherein the cell support matrix has a
21 shape selected from particles, tube, sponge, sphere, strand, coiled strand, capillary
22 network, film, fiber, mesh, and sheet.
- 23 23. A method of producing a tissue engineering construct, comprising:
24 providing a population of embryonic stem cells;
25 seeding the embryonic stem cells on a cell support matrix; and

- 1 exposing the embryonic stem cells to at least one agent selected to promote
2 differentiation of the stem cells along a predetermined cell lineage or into
3 a specific cell type,
4 wherein the step of exposing may be performed before or after the step of
5 seeding, or both.
- 6 24. The method of claim 23, wherein the embryonic stem cells are mammalian
7 embryonic stem cells.
- 8 25. The method of claim 24, wherein the embryonic stem cells are human embryonic
9 stem cells.
- 10 26. The method of claim 23, wherein the cell support matrix is three dimensional.
- 11 27. The method of claim 23, wherein a cross-sectional area of the matrix is not
12 reduced by more than 50% under a contractile force exerted by the embryonic
13 stem cells.
- 14 28. The method of claim 27, wherein a cross-sectional area of the matrix is not
15 reduced by more than 40% under a contractile force exerted by the embryonic
16 stem cells.
- 17 29. The method of claim 28, wherein a cross-sectional area of the matrix is not
18 reduced by more than 30% under a contractile force exerted by the embryonic
19 stem cells.
- 20 30. The method of claim 29, wherein a cross-sectional area of the matrix is not
21 reduced by more than 20% under a contractile force exerted by the embryonic
22 stem cells.
- 23 31. The method of claim 30, wherein a cross-sectional area of the matrix is not
24 reduced by more than 10% under a contractile force exerted by the embryonic
25 stem cells.

- 1 32. The method of claim 31, wherein a cross-sectional area of the matrix is not
2 reduced by more than 1% under a contractile force exerted by the embryonic stem
3 cells.
- 4 33. The method of claim 23, wherein the cell support matrix comprises a poly(lactic
5 acid) - poly(lactic acid-co-glycolic acid) mixture.
- 6 34. The method of claim 33, wherein the cell support matrix comprises a 50/50
7 mixture of poly(L-lactic acid) and poly(lactic acid-co-glycolic acid).
- 8 35. The method of claim 23, further comprising coating the cell support matrix with
9 an agent that promotes cell adhesion.
- 10 36. The method of claim 35, wherein the agent that promotes cell adhesion is selected
11 from fibronectin, integrins, and oligonucleotides that promote cell adhesion.
- 12 37. The method of claim 23, wherein the cell support matrix is biodegradable or non-
13 biodegradable.
- 14 38. The method of claim 23, wherein the cell support matrix is selected from PLA,
15 PGA, PLGA poly(anhydrides), poly(hydroxy acids), poly(ortho esters),
16 poly(propylfumerates), poly(caprolactones), polyamides, polyamino acids,
17 polyacetals, biodegradable polycyanoacrylates, biodegradable polyurethanes,
18 polysaccharides, polypyrrole, polyanilines, polythiophene, polystyrene,
19 polyesters, non-biodegradable polyurethanes, polyureas, poly(ethylene vinyl
20 acetate), polypropylene, polymethacrylate, polyethylene, polycarbonates,
21 poly(ethylene oxide), co-polymers of any of the above, adducts of any of the
22 above, and mixtures of any of the above polymers, co-polymers, and adducts with
23 one another.
- 24 39. The method of claim 23, further comprising adding one or more biomolecules,
25 small molecules, and bioactive agents to the cell support matrix.

- 1 40. The method of claim 23, further comprising disposing the embryonic stem cells
2 within a gel, wherein seeding the embryonic stem cells on the cell support matrix
3 includes disposing the gel on internal and external surfaces of the cell support
4 matrix.
- 5 41. The method of claim 40, wherein the gel is selected from collagen gel, alginate,
6 agar, and Growth Factor Reduced MATRIGELTM.
- 7 42. The method of claim 41, wherein the gel further comprises one or more of
8 laminin, fibrin, fibronectin, proteoglycans, glycoproteins, glycosaminoglycans,
9 chemotactic agents, and growth factors.
- 10 43. The method of claim 23, wherein culturing is conducted in a serum-free medium.
- 11 44. The method of claim 23, wherein the agent is selected from a growth factor, a
12 mechanical force, an electric voltage, a bioactive agent, a biomolecule, and a
13 small molecule.
- 14 45. The method of claim 44, wherein the growth factor is selected from cytokines,
15 eicosanoids, and differentiation factors.
- 16 46. The method of claim 45, wherein the growth factor is selected from activin-A
17 (ACT), retinoic acid (RA), epidermal growth factor, bone morphogenetic protein,
18 TGF- β , hepatocyte growth factor, platelet-derived growth factor, TGF- α , IGF-I
19 and II, hematopoietic growth factors, heparin binding growth factor, peptide
20 growth factors, erythropoietin, interleukins, tumor necrosis factors, interferons,
21 colony stimulating factors, fibroblast growth factors, nerve growth factor (NGF)
22 and muscle morphogenic factor (MMF).
- 23 47. The method of claim 44, wherein the mechanical force is selected from hoop
24 stress, shear stress, hydrostatic stress, compressive stress, tensile stress, and
25 combinations of the above.

- 1 48. The method of claim 23, wherein the cell support matrix has a shape selected
2 from particles, tube, sponge, sphere, strand, coiled strand, capillary network, film,
3 fiber, mesh, and sheet.
- 4 49. The method of claim 23, wherein providing includes culturing embryonic stem
5 cells in the presence of a growth factor.
- 6 50. The method of claim 49, wherein culturing is conducted in a serum-free medium.
- 7 51. A tissue engineering construct, comprising:
8 embryonic stem cells;
9 a three-dimensional cell support matrix comprising a 50/50 mixture of poly (L-
10 lactic acid) and poly (lactic-co-glycolic acid); and
11 TGF- β .
- 12 52. A tissue engineering construct, comprising:
13 embryonic stem cells;
14 a three-dimensional cell support matrix comprising a 50/50 mixture of poly (L-
15 lactic acid) and poly (lactic-co-glycolic acid); and
16 a member of activin A, IGF, and any combination of the above.
- 17 53. A tissue engineering construct, comprising:
18 embryonic stem cells;
19 a three-dimensional cell support matrix comprising a 50/50 mixture of poly (L-
20 lactic acid) and poly (lactic-co-glycolic acid); and
21 retinoic acid.
- 22 54. The tissue engineering construct of claim 51, 52, or 53, wherein the cell support
23 matrix further comprises one or more of fibronectin or growth factor-reduced
24 MATRIGEL.
- 25 55. A method of promoting tissue development, comprising:
26 providing a population of embryonic stem cells;

- 1 seeding the embryonic stem cells on a cell support matrix comprising a 50/50
2 mixture of poly(L-lactic acid) and poly(lactic-co-glycolic acid); and
3 exposing the embryonic stem cells to TGF- β ,
4 wherein the cells produce cartilaginous tissue.
- 5 56. A method of promoting tissue development, comprising;
6 providing a population of embryonic stem cells;
7 seeding the embryonic stem cells on a cell support matrix comprising a 50/50
8 mixture of poly(L-lactic acid) and poly(lactic-co-glycolic-acid); and
9 exposing the embryonic stem cells to one or more of activin A and IGF,
10 wherein the cells produce alpha feto protein and albumin.
- 11 57. A method of promoting tissue development, comprising:
12 providing a population of embryonic stem cells;
13 seeding the embryonic stem cells on a cell support matrix comprising a 50/50
14 mixture of poly (L-lactic acid) and poly (lactic-co-glycolic acid); and
15 exposing the embryonic stem cells to retinoic acid,
16 wherein the cells develop neuronal tissue structures.
- 17 58. The method of claims 55, 56, or 57 wherein the cell support matrix further
18 comprises one or more of fibronectin or Growth Factor-Reduced MATRIGELTM.
- 19 59. The method of claims 55, 56, or 57, wherein exposing comprises culturing the
20 seeded cell support matrix *in vitro* for two weeks and the method further
21 comprises implanting the seeded cell support matrix in an animal.
- 22 60. A method of promoting tissue development, comprising:
23 providing a population of embryonic stem cells;
24 seeding the embryonic stem cells on a cell support matrix;
25 culturing the seeded cell support matrix in the presence of a growth factor for a
26 predetermined amount of time; and
27 implanting the cultured cell support matrix in an animal.

- 1 61. The method of claim 60, wherein the cell support matrix is selected from PLA,
2 PGA, PLGA poly(anhydrides), poly(hydroxy acids), poly(ortho esters),
3 poly(propylfumerates), poly(caprolactones), polyamides, polyamino acids,
4 polyacetals, biodegradable polycyanoacrylates, biodegradable polyurethanes,
5 polysaccharides, polypyrrole, polyanilines, polythiophene, polystyrene,
6 polyesters, non-biodegradable polyurethanes, polyureas, poly(ethylene vinyl
7 acetate), polypropylene, polymethacrylate, polyethylene, polycarbonates,
8 poly(ethylene oxide), co-polymers of any of the above, adducts of any of the
9 above, and mixtures of any of the above polymers, co-polymers, and adducts with
10 one another.
- 11 62. The method of claim 60, wherein the three-dimensional cell support matrix
12 comprises a 50/50 mixture of poly (L-lactic acid) and poly (lactic-co-glycolic
13 acid).
- 14 63. The method of claim 60, further comprising coating the cell support matrix with
15 an agent that promotes cell adhesion.
- 16 64. The method of claim 63, wherein the agent that promotes cell adhesion is selected
17 from fibronectin, integrins, and oligonucleotides that promote cell adhesion.
- 18 65. The method of claim 60, further comprising disposing the embryonic stem cells
19 within a gel, wherein seeding the embryonic stem cells on the cell support matrix
20 includes disposing the gel on internal and external surfaces of the cell support
21 matrix.
- 22 66. The method of claim 65, wherein the gel is selected from collagen gel, alginate,
23 agar, and Growth Factor Reduced MATRIGELTM.
- 24 67. The method of claim 65, wherein the gel further comprises one or more of
25 laminin, fibrin, fibronectin, proteoglycans, glycoproteins, glycosaminoglycans,
26 chemotactic agents, and growth factors.

- 1 68. The method of claim 60, wherein the growth factor is selected from activin-A
2 (ACT), retinoic acid (RA), epidermal growth factor, bone morphogenetic protein,
3 TGF- β , hepatocyte growth factor, platelet-derived growth factor, TGF- α , IGF-I
4 and II, hematopoietic growth factors, heparin binding growth factor, peptide
5 growth factors, erythropoietin, interleukins, tumor necrosis factors, interferons,
6 colony stimulating factors, fibroblast growth factors, nerve growth factor (NGF)
7 and muscle morphogenic factor (MMF).
- 8 69. The method of claim 60, wherein the predetermined period of time is two weeks.
- 9 70. The method of claim 60, wherein culturing is conducted in a serum-free medium.